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AUTHOR(S):

Murakami, Akira

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Dose-dependent functionality and toxicity of green tea polyphenols in experimental rodents

Akira Murakami

Division of Food Science and Biotechnology

Graduate School of Agriculture

Kyoto University, Kyoto, 606-8502, Japan

TEL: +81-75-753-6282; FAX: +81-75-753-6284; Email: cancer@kais.kyoto-u.ac.jp

Abstract

A large number of physiologically functional foods are comprised of plant polyphenols. Their antioxidative activities have been intensively studied for a long period and proposed to be one of the major mechanisms of action accounting for their health promotional and disease preventive effects. Green tea polyphenols (GTPs) are considered to possess marked anti-oxidative properties and versatile beneficial functions, including anti-inflammation and cancer prevention. On the other hand, some investigators, including us, have uncovered their toxicity at high doses presumably due to pro-oxidative properties. For instance, both experimental animal studies and epidemiological surveys have demonstrated that GTPs may cause hepatotoxicity. We also recently showed that diets containing high doses (0.5-1%) of a GTP deteriorated dextran sodium sulfate (DSS)-induced intestinal inflammation and carcinogenesis. In addition, colitis mode mice fed a 1% GTP exhibited symptoms of nephrotoxicity, as indicated by marked elevation of serum creatinine level. This diet also increased thiobarbituric acid-reactive substances, a reliable marker of oxidative damage, in both kidneys and livers even in normal mice, while the expression levels of antioxidant enzymes and heat shock proteins (HSPs) were diminished in colitis and normal mice. Intriguingly, GTPs at 0.01% and 0.1% showed hepato-protective activities, *i.e.*, they significantly suppressed DSS-increased serum aspartate aminotransferase and alanine

aminotransferase levels. Moreover, those diets remarkably restored DSS-down-regulated expressions of heme oxygenase-1 and HSP70 in livers and kidneys. Taken together, while low and medium doses of GTPs are beneficial in colitis model mice, unwanted side-effects occasionally emerge with high doses. This dose-dependent functionality and toxicity of GTPs are in accordance with the concept of hormesis, in which mild, but not severe, stress activates defense systems for adaptation and survival.

Keywords: green tea polyphenol; colitis; nephrotoxicity; heat shock protein; molecular chaperone; pro-oxidation

Abbreviations: ABC, ATP-binding cassette; ACR, acrolein; ALS, amyotrophic lateral sclerosis; ALT, alanine aminotransferase; AST, aspartate aminotransferase: DMH, dimethylhydrazine; DSS, dextran sulfate sodium; EGCG, (-)-epigallocatehin-3-gallate; ERK, extracellular signal-regulated kinase; GTP, green tea polyphenols; GSH, glutathione; GST, GSH *S*-transferase; HNE, 4-hydroxy-2-nonenal; HO-1, heme oxygenase-1; Hsf, heat shock factor; HS, heat shock; HSP, heat shock protein; Keap1, Kelch-like ECH-associated protein; MDA, malondialdehyde; MMP, matrix metalloproteinase; Nrf2, nuclear factor (erythroid-derived 2)-like 2; NQO1, NAD(P)H dehydrogenase; PQC, protein quality control;

ROS, reactive oxygen species; SOD, superoxide dismutase; TBARS, thiobarbituric acid-reactive substances; Ub, ubiquitin

Stress-induced biomolecule modifications

Homeostatic regulation of biological functions of macromolecules, such as membrane lipids, enzymes, proteins, and DNA, is a key determinant for health promotion, disease prevention, and longevity. Reactive oxygen species (ROS) have long been demonstrated to play numerous roles in both physiological and pathological conditions *via* oxidative modification of biomolecules present in cells and tissues. Accumulation of oxidative damage footprints in those biological molecules has been described to be involved in the onset of numerous diseases and aging [1]. In particular, biochemical modifications of cellular DNA [2] and proteins [3] by ROS and lipid peroxidation products, such as acrolein [4], malondialdehyde [5], and 4-hydroxy-2-nonenal [6] lead to either loss or undesired gain of their functions for homeostasis disruption (Figure 1). In addition, mitochondrial DNA mutations caused by ROS play significant roles in dysfunctions of cellular metabolism, and defense and other systems [7], though Laqouge and Larsson have proposed that those mutations may be generated by replication errors rather than accumulated oxidative damage [8]. Recently, Akatsuka *et al.* uncovered a novel base modification, *i.e.*, 1,N^6-propanoadenine (acrolein-adenine), in a ferric nitrilotriacetate-treated mouse model, in

which oxidative stress plays an essential role in the development of renal carcinogenesis [9]. Also, mutations of the *Cu,Zn-sod* gene were reported to partially truncate anti-oxidative systems, thereby increasing the risk of familial amyotrophic lateral sclerosis (ALS) [10], a neurodegenerative disease that exhibits dysfunctions of muscle movement and strength. On the other hand, proteo-stresses have been shown to be characterized to be inducible by physical, chemical, and biological stimuli, and interfere with and disable protein folding for denaturation [11]. Other recent studies have demonstrated that accumulation of un- and mis-folded proteins may promote the formation of their aggregates, the hallmark of neurodegenerative diseases, such as Alzheimer's, Parkinson's and Huntington's diseases [12]. In addition, the status of cellular junk proteins is associated with adiposity and thus the development of metabolic syndrome [13]. Furthermore, it should be pointed out that concerted and tight regulation of the biological protein quality may contribute to longevity, as demonstrated in experiments using the naked mole rats, which have marked protein stability and a long life-span [14]. Thus, it is highly conceivable that quality maintenance of biological proteins greatly affects health status and longevity. Among a number of constitutive and inducible mechanisms that function to protect biological macromolecules from oxidative stress and proteo-stress, 2 representative biochemical defense systems are highlighted (Figure 2) and described below.

The Keap1/Nrf2-dependent defense system

Anti-oxidation can be defined as a self-defense mechanism ubiquitously distributed among organisms. Anti-oxidative modes of actions can be classified into at least the following 3 categories: (1) scavenging and quenching ROS [as seen with vitamin C, vitamin E, glutathione (GSH), *etc.*], (2) attenuation of ROS-generating enzymes (NADPH oxidase, xanthine oxidase, *etc.*), and (3) up-regulation of anti-oxidant enzymes [superoxide dismutase (SOD), catalase, *etc.*]. The Kelch-like ECH-associated protein 1/nuclear factor (erythroid-derived 2)-like 2 (Keap1/Nrf2) system, belonging to the category (3), adaptively functions to protect cells from endogenous and exogenous oxidative and electrophilic damages [15] (Figure 3). In a normal state, the transcription factor Nrf2 is continuously ubiquitinated by the Cul3-Keap1 ubiquitin E3 ligase complex and thereby rapidly transported to degradation systems in proteasomes. ROS and electrophilic chemicals oxidize the reactive cysteine residues of Keap1 in both direct and indirect manners. This critical step stabilizes Nrf2, thereby inducing robust expressions of a battery of cytoprotective genes, including those related to anti-oxidation [Cu/Zn-SOD, Mn-SOD, extracellular SOD, γ-glutamylcysteine synthetase, *etc.*], xenobiotic detoxification [GSH *S*-transferase (GST), glucuronidase, sulfatase, *etc.*], and protein quality control (PQC) (molecular chaperones,

ubiquitin/proteasome systems, *etc.*) for adaptation [15]. In addition, prior to translocation of Nrf2 into the nucleus, its transcription activity is modulated by several protein kinases, which are simultaneously activated by stressors. Feng *et al*. disclosed that activation of Akt and extracellular signal-regulated kinase (ERK)1/2 is required for activation of Nrf2, leading to up-regulation of the expression of heme oxygenase (HO)-1, one of the major inducible anti-oxidant enzymes [16]. On the other hand, Nrf2 activity is continuously repressed in a normal state by Bach1, which constitutively binds to the Maf recognition element [17].

Heat shock proteins as molecular chaperones

Various types of stresses toward biological proteins critically disrupt their conformation and folding state, which often results in abolishment of their biological functions. A number of recent studies have indicated that several distinct PQC systems play key roles in counteraction against proteo-stress. Heat shock proteins (HSPs), highly conserved families of proteins ubiquitously expressed in most types of cells, are molecular chaperones that allow misfolded and unfolded proteins to achieve functionally active conformation (Figure 4). The expression and functional status of HSPs are considered to be critical determinants of PQC systems and thus essentially associated with homeostasis, health and longevity. In fact, expression of HSPs at high levels substantially contributes to

extending the lifespan of many experimental animals [18]. HSPs are comprised of numerous family proteins. Constitutive HSPs, sharing approximately 1% of cytosolic proteins, are essential for maintaining PQC systems under a non-stressed condition. On the other hand, various types of stressors are known to up-regulate inducible HSPs. In a normal state, HSP90β, a major constitutive isoform, is bound to the transcription factor heat shock factor1 (Hsf1), thereby forcing it to be biologically dormant [19]. Heat shock and some other stimuli are capable of dissociating this heterodimer complex. Thereafter, the resultant free Hsf1 forms a trimer complex and is also phosphorylated at multiple sites, finally translocating into the nucleus to induce a number of HSP genes to amplify defense capacity [19]. For example, HSP70, a major inducible isoform, protects neurons from protein aggregation and apoptosis, which may contribute to regulate Parkinson's, Alzheimer's and polyglutamine diseases, and ALS [20]. Additionally, Hsf1 has been reported to up-regulate ABC transporters [21] and Bcl-2 [22], both of which potentiate cellular defense capacity against foreign chemicals and other stress stimuli. Meanwhile, it is interesting to note that there are mechanistic interactions between oxidative stress- and proteo-stress-triggered signaling pathways. In this regard, Back *et al*. showed that oxidative stress chemically cleaves HSP90 protein, resulting in dissociation with and subsequent degradation of its client proteins, including Bcr-Abl, RIP, and Akt, for cancer cell death [23]. Also, oxidative stress-triggered

Keap1/Nrf2 system activation was reported to up-regulate the expression of p62, a multifunctional adapter protein implicated in autophagy against proteo-stress [24]. The crosstalk between oxidative stress and proteo-stress-triggered signaling pathways resembles that between the complement system and toll-like receptors in innate immunity [25], and is critical for first-line host defense.

Potential side-effects of green tea polyphenols

Introduction

Tea (*Camellia sinensis*, Theaceae) is a popular beverage and consumed by over two-thirds of the world population [26]. Green tea polyphenols (GTPs) account for 30-42 % of the dry weight of the solids in brewed green tea, while (-)-epigallocatehin-3-gallate (EGCG) (Figure 5A) is estimated to account for 50-80% of the total catechins in tea [26]. There is scant evidence that oral consumption of green tea, but not supplements, even at large amounts has side-effects, and is actually beneficial. For example, an epidemiological survey, of over 8500 people followed up for 9 years showed that the age-standardized average annual incidence rate of cancer was significantly lower among females who daily consumed 10 or more cups of green tea [45]. In addition to the health benefit effects of GTPs [27-29], many independent studies have also suggested their harmful aspects, which

are largely due to pro-oxidative properties when consumed at high doses. These pro-oxidative activities are broadly attributable to their catechol structures (*ortho* diphenols, Figure 5B). This functional moiety has a chemical characteristic to generate superoxide anion radical (O_2^-) from molecular oxygen ($^3O_2$) through formation of an electrophilic *o*-quinone counterpart. In addition, Kajiya *et al.* hypothesized that an ester group in EGCG plays a crucial role in biochemical interactions with the lipid bilayer, providing it with a high affinity to the cell membrane [30]. Of note, GTPs covalently bind protein thiols through their conversion to an *o*-quinone counterpart [31, 32], while Elbling and colleagues showed that EGCG exhibited a pro-oxidative, but not anti-oxidative, property in cellular models [33]. Similarly, EGCG induced injury in mouse blastocysts through intrinsic apoptotic signaling processes to impair sequent embryonic development [34]. We also found that GTPs increased expression of the tumor metastatic protein, pro-matrix metalloproteinase-7 (pro-MMP-7), in human colon adenocarcinoma cells *via* a pro-oxidation-dependent mechanism since both SOD and catalase suppressed the production of pro-MMP-7 [35]. However, the pro-oxidative activity of GTPs may not be recognized as not necessarily harmful because it executes induction of cancer cell apoptosis [36]. Moreover, as indicated above, pro-oxidative stimuli may activate the Keap1/Nrf2 systems for exhibiting anti-oxidative, anti-inflammatory, and chemopreventive activities [37]. In fact, Na *et al.*

showed that EGCG acted as a pro-oxidant to activate Nrf2-dependent gene expressions of some representative antioxidant enzymes [38, 39]. Thus, it can be argued that the pro-oxidative nature of GTPs cannot be simply defined as harmful, and the risks and benefits depend on the situation. Meanwhile, the anti- and pro-oxidative properties of GTPs as well as many other polyphenols are determined by their doses, the presence or absence of oxidants, the responsiveness of adaptation systems, and other factors.

Intestinal toxicity

Ulcerative colitis encompasses several chronic inflammatory disorders that cause damage to the gastrointestinal tract and affect individuals of both sexes throughout life [46]. Dextran sulfate sodium (DSS)-induced colitis animal models are most frequently used in studies of inflammatory bowel disease. Importantly, a DSS-induced model exhibited many symptoms similar to those seen in human ulcerative colitis, including diarrhea, bloody feces, body weight loss, mucosal ulcerations, and shortening of the colorectum [47]. We previously examined the effects of the commercially available GTPs (Figure 5C) (0.01%-1% in the basal diet) on dimethylhydrazine (DMH)-initiated DSS-promoted mouse colorectal carcinogenesis, and found that concentrations of 0.1%, 0.5% and 1%, but not 0.01%, tended to increase tumor formation [48]. Furthermore, 1% GTP treatment following the DMH

exposure tended to increase histological tumor formation as compared to DMH alone group. Also, the expressions of interleukin-1β and macrophage-migration inhibitory factor, both of which play essential roles in early pro-inflammatory processes, were significantly increased in the DSS + 0.5% GTP and DSS + 1% GTP groups when compared with the DSS alone group. These findings are in line with those reported by Hirose *et al*., who demonstrated that dietary GTPs enhanced colon carcinogenesis in rats treated with DMH [49]. Moreover, consumption of a 0.36% GTP diet resulted in a significant increase in multiplicity of colonic tumors as compared to the carcinogen azoxymethane alone [50].

Hepatic and renal toxicity

A recent study found that repeated administrations of high-dose GTPs (750 mg/kg) increased hepatic injuries, which were augmented under febrile conditions induced by endotoxin [40]. Likewise, a single high dose of EGCG (1500 mg/kg) dramatically increased plasma alanine aminotransferase (ALT) and reduced survival in male CF-1 mice [41]. In addition, a green tea extract potentiated acetaminophen-induced hepatotoxicity in mice at doses of 500 and 1000 mg/kg [42]. To support those experimental findings with cultured cells and rodents, an epidemiological survey based on an extensive publication analysis between 1999 and 2008 by Mazzanti *et al.* showed a causal association between green tea

supplement intake and liver damage [43]. These authors speculated that this hepatotoxicity is probably due to EGCG or its metabolites which can induce oxidative stress in the liver under conditions related to the metabolism of patient [43]. Furthermore, Yellapu *et al*. implied the side-effects of 'fat burners' and dietary supplements, including green tea constituents, which may be hepatotoxic and may not be free of adverse effects [44]. Collectively, ingestion of high doses of GTPs and other related supplements may increase the risk of hepatic injury because of their pro-oxidative properties, though precise mechanisms underlying that toxicity have not been fully elucidated.

We also assessed the potential toxicity of GTP toward liver and kidney functions using a DSS-induced colitis model because, as mentioned above, high-dose GTPs may show harmful effects in those organs. Importantly, oral feeding of 1% GTPs, caused kidney dysfunctions in DSS-treated ICR mice, as revealed by increases in serum creatinine, the most reliable biomarker of nephropathy [51]. In addition, we observed that the amounts of thiobarbituric acid-reactive substances (TBARS, a potential indicator of lipid peroxidation) were increased in both the livers and kidneys of the 1% GTP group. Previous pharmacological studies have shown that EGCG is metabolized through methylation, glucuronidation, and sulfation under normal physiological conditions, and then subsequently excreted in urine [52]. It is important to note that, most, if not all, metabolites are biologically

inactivated and thus much less toxic than the intact form of EGCG. Furthermore, these bioactive electrophiles have a potential to bind to nucleophilic amino acid residues (*e.g.*, cysteine) for inducing proteo-stress [53, 54], and those stress-generating properties may be associated with their toxicity in the liver and kidneys. Meanwhile, in other studies high-dose EGCG reduced the expressions of Nrf2-dependent antioxidant enzymes, including HO-1, SOD, NAD(P)H quinone oxidoreductase 1, and catalase [48, 55], and some of those findings showing positive correlations with our results [48, 51]. Importantly, Nrf2 activity is a primary contributor to hepatoprotection [56]. Also, Lim and colleagues have found a correlation between decreased SOD activity and aggravated diabetic nephropathy [57], while HO-1 was reported to attenuate the progression of chronic kidney disease [58]. Therefore, we speculate that high-dose GTPs not only act in a pro-oxidant manner, but also down-regulate Nrf2-dependent antioxidant enzymes for disordering homeostasis in the liver and kidney. Downregulation of HSP70 in the colonic mucosa of DSS-exposed mice has been found to have an association with intestinal inflammation [59]. It should also be noted that renal and hepatic levels of HSP27 were remarkably down-regulated by 1% GTP administration as compared with the DSS group [51], while more strikingly, HSP90 in the kidneys and livers of the non-treated mice was notably down-regulated in mice that received the 1% and even by 0.1% GTP diets. These results are consistent with those of Tran *et al.*,

who previously found that HSP90 was repressed by EGCG in MCF-7 human breast cancer cells [60]. As noted above, HSP90 is the most abundant molecular chaperone and plays pivotal roles in maintaining organ homeostasis [61]. Therefore, a 1% GTP diet might induce hepatic and renal toxicity by attenuating, at least in part, the expressions of anti-oxidant enzymes (SOD, HO-1, *etc*.) and molecular chaperones (HSP90, HSP27, *etc*).

Beneficial effects of GTPs at low and medium doses

In contrast to high doses, medium- and low-dose GTP diets (0.01-0.1%) significantly inhibited rat colon carcinogenesis [62], which correlated in part with our previous findings [48], *i.e*., a diet of 0.01%, but not 0.5% or 1%, GTP tended to decrease tumor multiplicity or frequency. Thus, administration of GTPs exhibits both beneficial and harmful effects on colon carcinogenesis, depending on the dose. In parallel, Kohlmeier *et al*. reported that it is difficult to define the inhibitory effects of green tea consumption on colon cancer development in humans [63]. Interestingly, medium and low doses of GTPs also exhibited beneficial effects on hepatic and kidney functions, as GTPs at 0.01% and 0.1% significantly suppressed DSS-induced elevation of serum aspartate aminotransferase and ALT levels [64]. Similarly, GTPs at both 0.01% and 0.1%, but not 1%, remarkably up-regulated the expressions of HO-1 and HSP70 in livers and kidneys of DSS-exposed mice.

Collectively, low- and medium-doses of GTPs have beneficial effects on DSS-induced intestinal inflammation and carcinogenesis as well as hepatotoxicity and nephrotoxicity *via* up-regulation of self-protective enzymes, while those are abolished with a high dose (Figure 6).

GTPs are potential phytoalexins (plant toxins)

In nature, plants have a critical disadvantage for survival as compared with animals, as they are unable to move to avoid biological enemies and stress stimuli, such as invading microorganisms and insects, herbivorous animals, and intense sunlight. Thus, they have developed specific biological systems that can adapt to and counteract against various stresses. In particular, phytochemicals, biosynthesized in both constitutive and inducible manners, function as a central group to fight against and mitigate exogenous stresses. This raises an important question as to why phytochemicals, biosynthesized for their adaptation, exhibit health benefit functions in animals, including humans. It is reasonable to speculate that those chemicals have no nutritional value and are xenobiotics substances in animals. Although mammals efficiently and actively absorb and utilize primary products, including sugars, protein, and lipids, as essential nutrients, the bioavailability of phytochemicals in experimental rodents and humans has been consistently reported to be fairly poor in several

studies. For example, a bioavailability study showed that administration of EGCG resulted in its substantial biotransformation, *e.g.*, glucuronidation, sulfation, and *O*-methylation, and its blood concentrations were quite limited [65, 66]. Therefore, most, if not all, phytochemicals are substantially foreign chemicals to mammals, and it is not surprising that they are actively subjected to detoxification and excretion systems. Based on those findings, it is reasonable that phytochemicals up-regulate anti-oxidant enzymes, xenobiotic detoxification proteins, and molecular chaperones, all of which are recognized as defense molecules against oxidative- and proteo-stresses induced by those chemicals. Of paramount importance, some of those adaptive responses have mechanistic links to their already-known, beneficial bioactivities [67, 68]. Exposure levels to those foreign chemicals must essentially remain at mild ranges to gain beneficial effects, otherwise, no positive effects or catastrophic outcomes would become dominant. This notion is in line with the concept of hormesis [69-71], in which mild biological or physical stresses at mild levels activate defense systems for adaptation (Figure 7).

Conclusion

High doses, but not low and medium doses, of dietary GTPs deteriorated colitis and colorectal cancer, and also induced liver and kidney dysfunctions in mice. The fundamental

reason why phytochemicals trigger activation of self-defense systems is probably because they are essentially foreign chemicals with no nutritional value mammals. Accumulated evidence regarding the functionality and toxicity of phytochemicals reminds us of the saying 'More than enough is too much'.

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Disclosure

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Figure legends

Figure 1

Modifications of cellular membrane lipids, proteins, and DNA by reactive oxygen species and electrophilic aldehyde compounds, the latter of which are degradation products of lipid peroxide. ROS, reactive oxygen species; ACR, acrolein; MDA, malondialdehyde; HNE, 4-hydroxy-2-nonenal; HS, heat shock

Figure 2

Adaptive defense systems for oxidative stress and proteo-stress, which are specifically sensed by Keap1 and HSP90β, respectively, for activating the key transcription factors, Nrf2 and HSF1 to induce robust expressions of numerous genes for adaptation. Nrf2, nuclear factor (erythroid-derived 2)-like 2; SOD, superoxide dismutase; HO-1, heme oxygenase-1;

GST, glutathione *S*-transferase; NQO1, NAD(P)H dehydrogenase 1; HSP, heat shock protein; HSF1, heat shock factor1; ABC, ATP-binding cassette

Figure 3

Mechanistic scheme for Nrf2 activation and resultant expressions of self-defense genes. Ub, ubiquitin; ERK, extracellular signal-related kinase

Figure 4

Proteo-stress-induced HSF1-dependent activation of HSP40 and HSP70 protein expressions for increased proteo-stress resistance. HSP, heat shock protein; Hsf1, heat shock factor1; HSE, heat shock element

Figure 5

(A) Chemical structure of EGCG, (B) formation of *o*-quinone counterpart and its reaction with protein thiols to form a covalent bond, (C) composition of GTPs used in our experiments

Figure 6

Summary of results of experiments on effects of low medium, and high doses of GTPs on large intestine, liver, and kidneys of ICR mice. Summarized from ref. [50] and [63]. TRABS, thiobarbituric acid reactive substances; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HSP, heat shock protein; HO-1, heme oxygenase-1

Figure 7

Hormetic curve illustrating the relationship between the stress intensity (dose) and homeostatic capacity. The hormetic zone areas differ among the various stressors, with a more frequent chance of adaptation with a larger zone

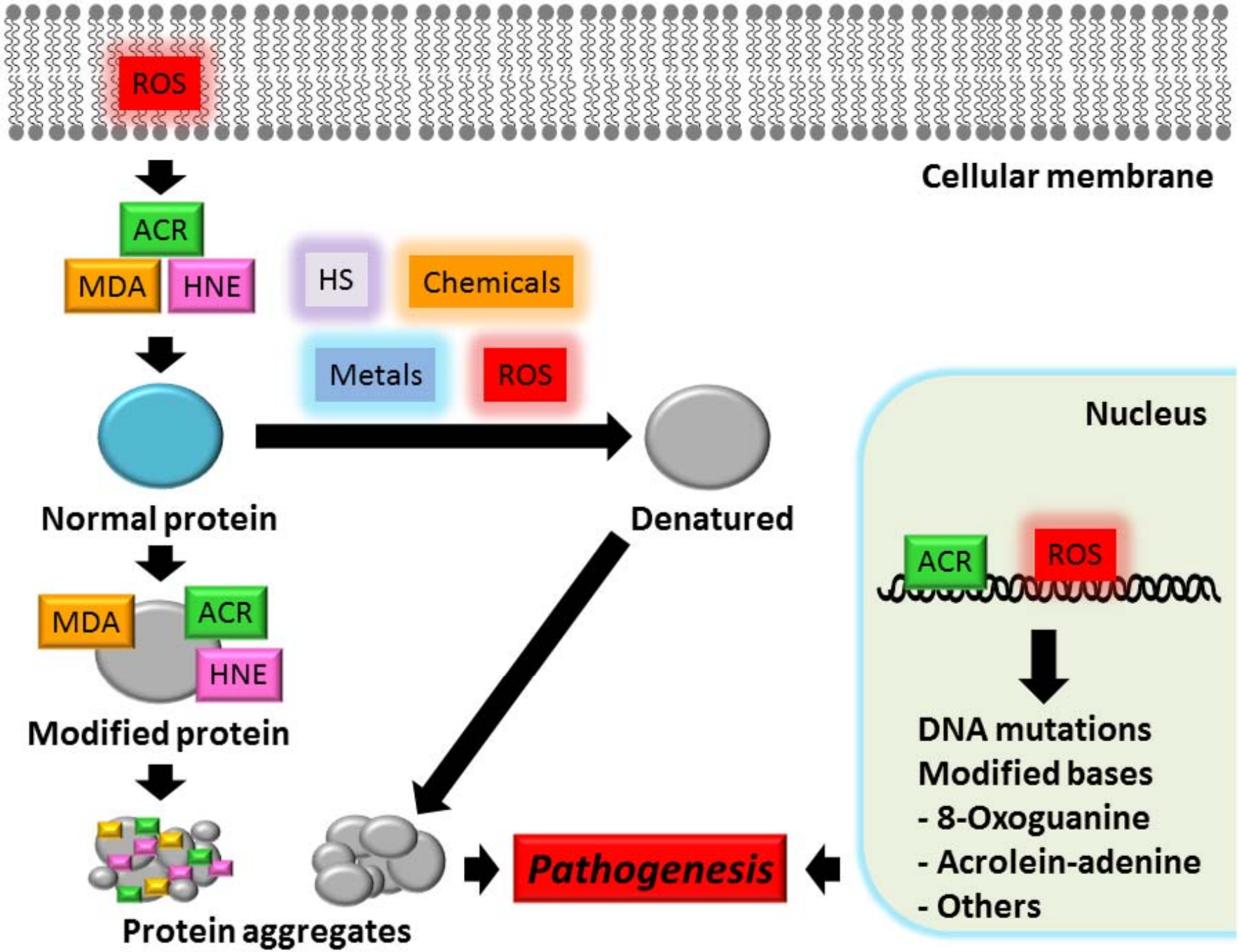

ROS
Cellular membrane
ACR
MDA
HNE
HS
Chemicals
Metals
ROS
Normal protein
Denatured
Nucleus
ACR
ROS
MDA
ACR
HNE
Modified protein
DNA mutations
Modified bases
- 8-Oxoguanine
- Acrolein-adenine
- Others
Pathogenesis
Protein aggregates

Figure 1

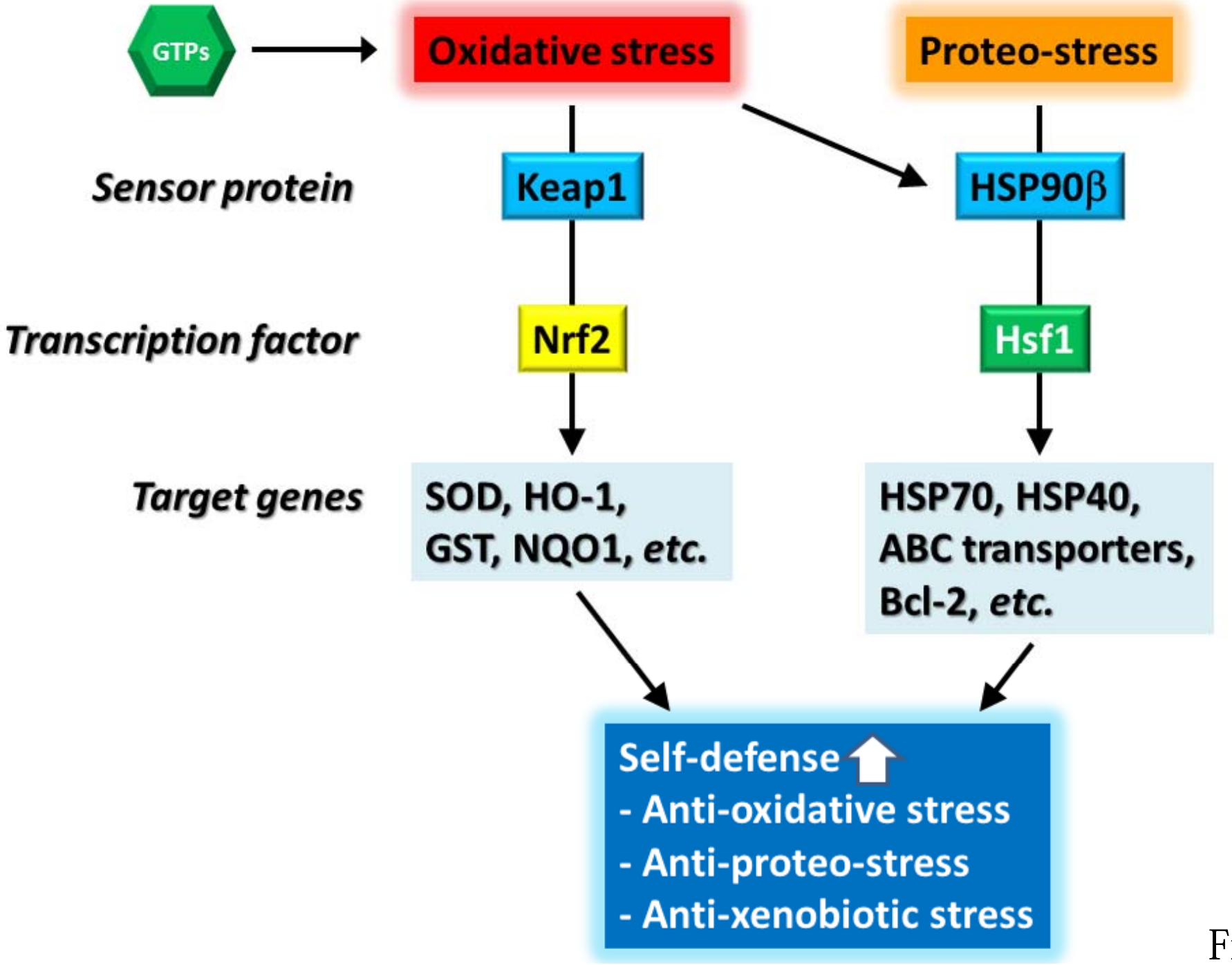

GTPs
Oxidative stress
Proteo-stress
Sensor protein
Keap1
HSP90β
Transcription factor
Nrf2
Hsf1
Target genes
SOD, HO-1,
GST, NQO1, etc.
HSP70, HSP40,
ABC transporters,
Bcl-2, etc.
Self-defense
- Anti-oxidative stress
- Anti-proteo-stress
- Anti-xenobiotic stress

Figure 2

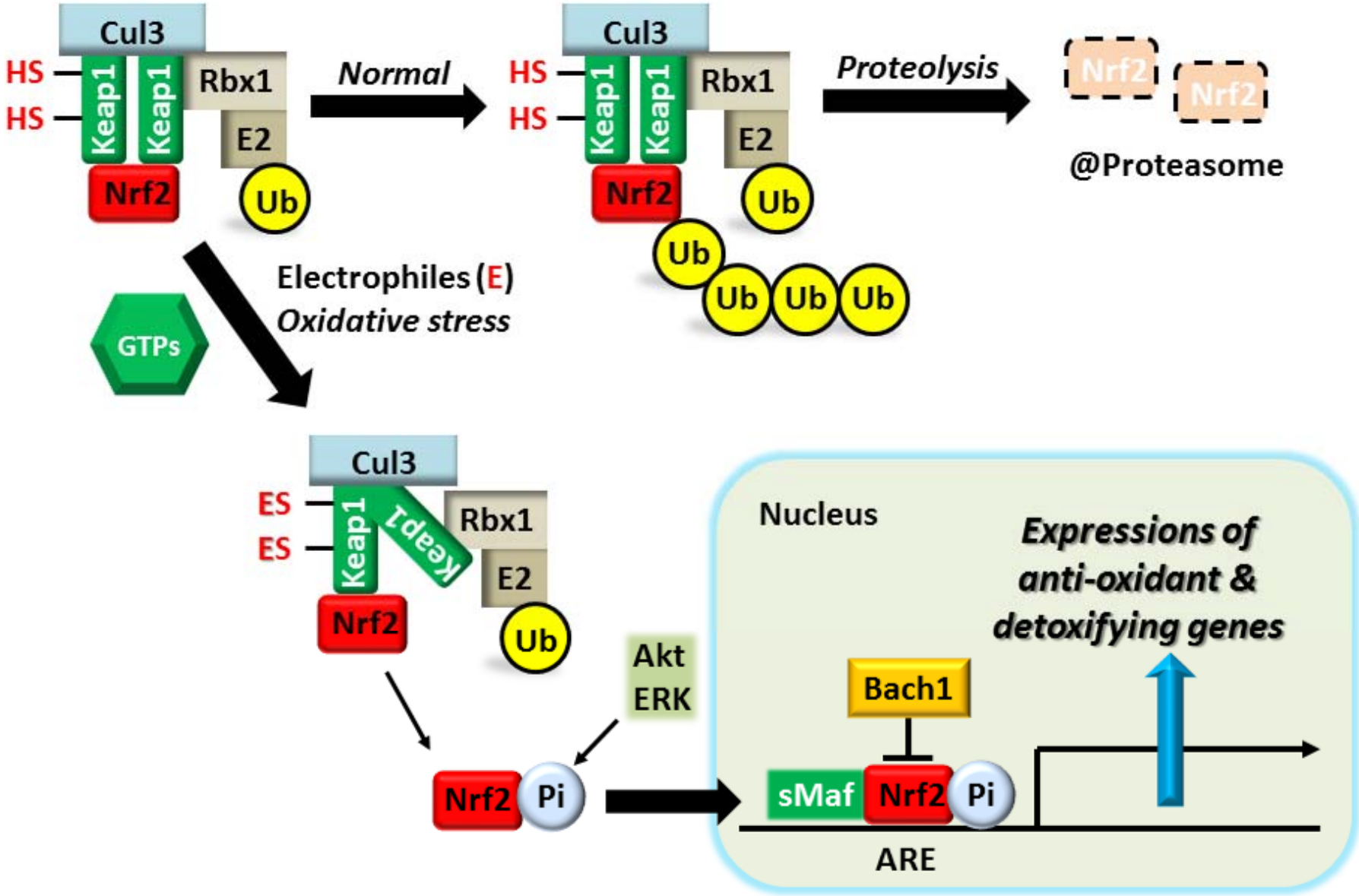

Cul3
HS
HS
Keap1
Keap1
Rbx1
E2
Nrf2
Ub
Normal
Proteolysis
Nrf2
Nrf2
@Proteasome
Ub
Ub
Ub
Ub
Ub
Electrophiles (E)
Oxidative stress
GTPs
ES
ES
Keap1
Keap1
Akt
ERK
Nucleus
Expressions of
anti-oxidant &
detoxifying genes
Bach1
sMaf
Nrf2
Pi
ARE

Figure 3

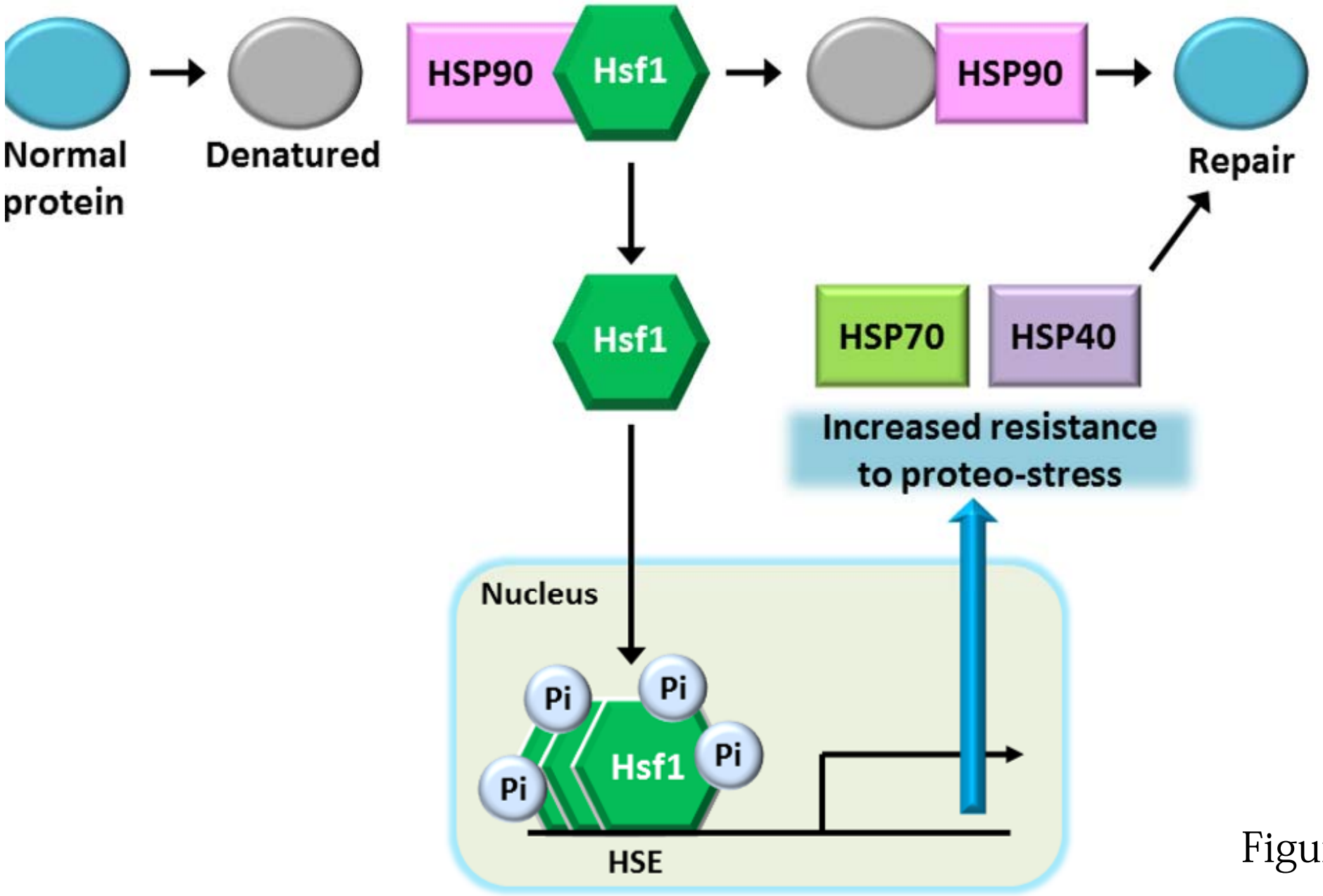
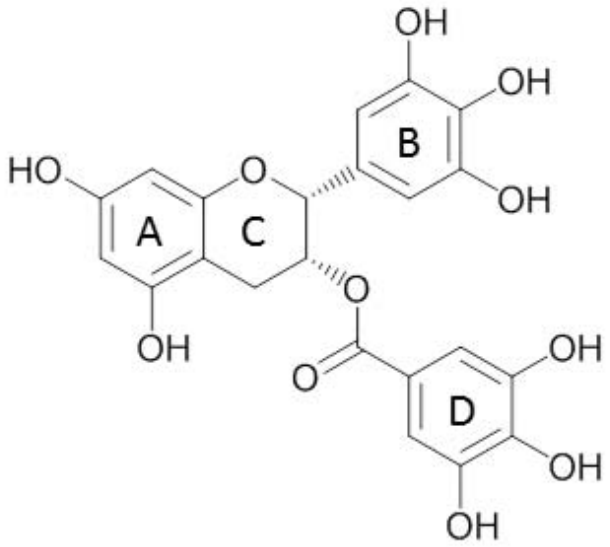

Normal protein
Denatured
HSP90
Hsf1
HSP90
Repair
Hsf1
HSP70
HSP40
Increased resistance to proteo-stress
Nucleus
Pi
Pi
Pi
Hsf1
Pi
HSE

Figure 4

A



C

Component	%
Polyphenols	95
Catechins	70
EGCG	35
Caffeine	3
Water	5

B

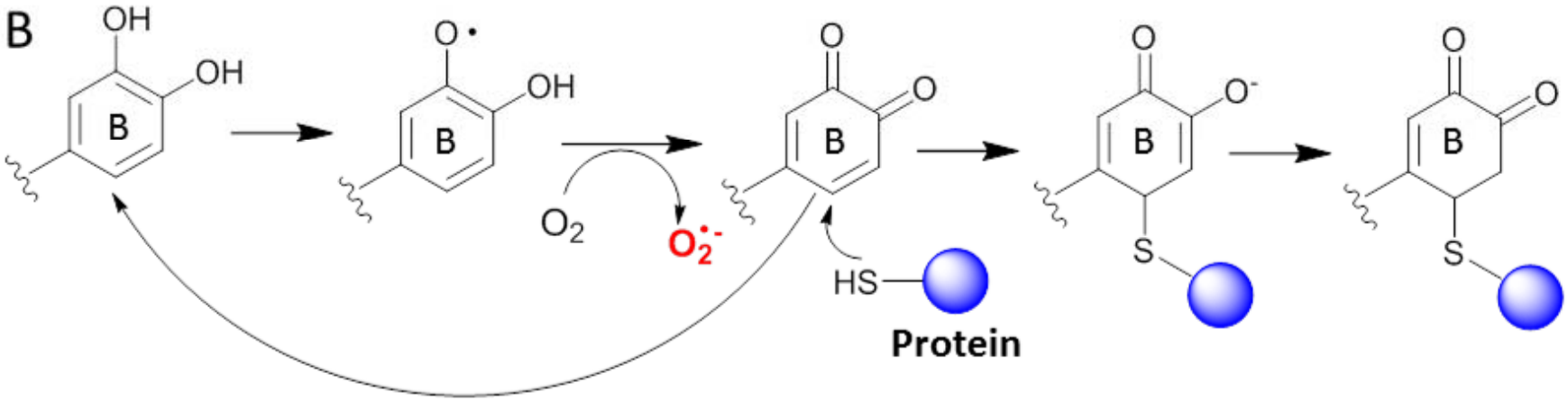


Figure 5

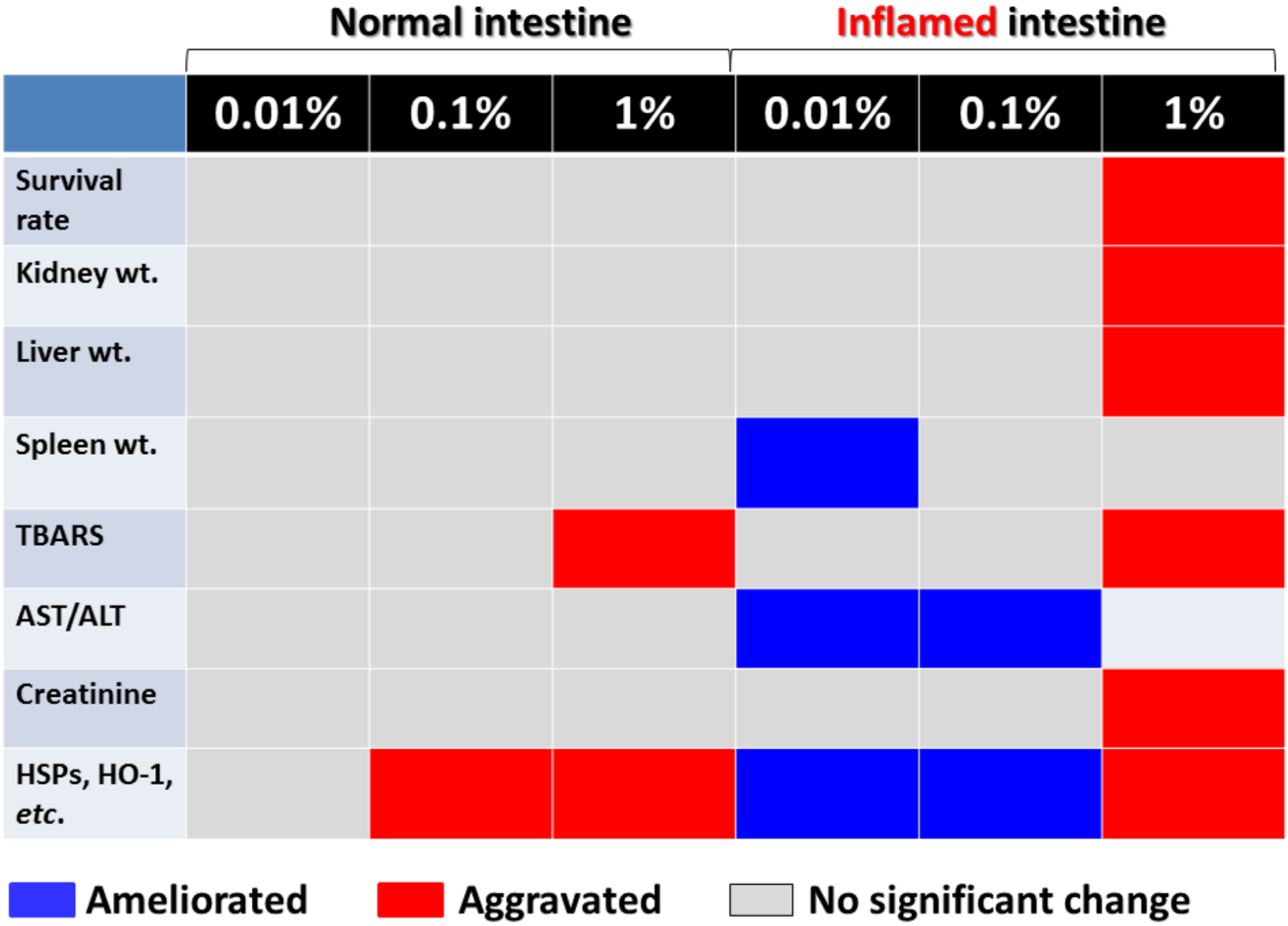

Normal intestine
Inflamed intestine
0.01%
0.1%
1%
0.01%
0.1%
1%
Survival rate
Kidney wt.
Liver wt.
Spleen wt.
TBARS
AST/ALT
Creatinine
HSPs, HO-1, etc.
Ameliorated
Aggravated
No significant change

Figure 6

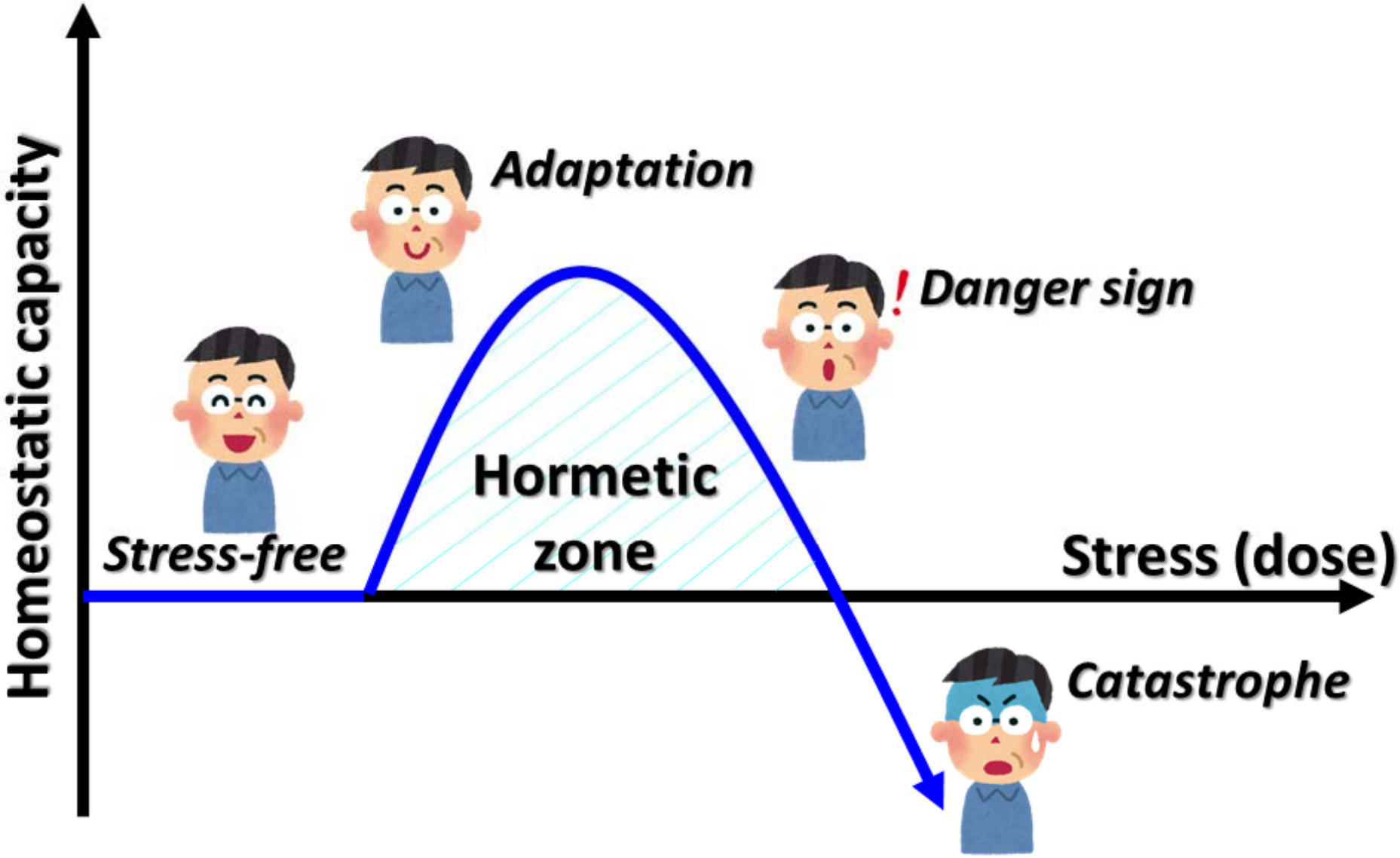

Homeostatic capacity
Adaptation
! Danger sign
Hormetic
zone
Stress-free
Stress (dose)
Catastrophe

Figure 7